ABSTRACTS – ORAL

801 Vascular Growth and Function

Monday, March 30, 1998, 10:30 a.m.–Noon
Georgia World Congress Center, Room 261W

10:30

801-1 Pravastatin Activates Endothelial Nitric Oxide Synthase Independent of Its Lipid Lowering Actions

W.H. Kaesemeyer, R.B. Caldwell, T.A. Taylor, J. Huang, R.W. Caldwell
Department of Pharmacology & Toxicology and Vascular Biology Center, Medical College of Georgia, Augusta, GA 30912, USA

Background: Recent studies with pravastatin (PRA) have suggested that reductions in myocardial infarction and stroke are not totally explained by reductions in LDL cholesterol (LDL). A study comparing the effects of PRA vs. simvastatin showed that only PRA inhibited platelet aggregation with no difference in actions between the agents on cholesterol levels. Since nitric oxide (NO) is a well known inhibitor of platelet aggregation, we investigated the possibility that PRA is an agonist of endothelial nitric oxide synthase (eNOS) independent of its lipid lowering actions.

Methods: NO production was measured by conversion of hemoglobin to methemoglobin and by use of a NO sensitive electrode using isolated bovine aortic endothelial cells in culture. Responses to acetylcholine (ACH) and PRA for 3 min were measured in the presence and absence of nitro-L-ARG methyl ester (L-NAME, 10^{-3} M) and then excess L-ARG (10^{-3} M).

Results: ACH and PRA (both at 10^{-6} and 10^{-5} M) stimulated NO production to 227 & 521 and 185 & 413 nmoles · min, respectively. L-NAME reduced these responses to 39 & 56 and 30 & 42% of initial values, respectively. Excess L-ARG increased NO production to 134 & 149 and 123 & 133% of initial values, respectively. These results were confirmed by NO measurements obtained with an NO electrode.

Conclusion: PRA can stimulate eNOS activity. Clinical results seen with PRA not totally explained by LDL reductions may be the result of an independent action of PRA on eNOS activation.

10:45

801-2 Direct Demonstration of Nitric Oxide Formation From Purified Nitric Oxide Synthase

Y. Xia, J.L. Zweier *Johns Hopkins University School of Medicine, Baltimore, MD, USA*

Although nitric oxide (NO), a central mediator of cardiovascular regulation, is widely considered to be derived from NO synthase (NOS), direct demonstration of NO formation from the purified enzyme has been lacking. Based on failure to detect NO from isolated NOS, it was recently reported that NOS does not synthesize NO, but rather generates nitroxyl anion (NO^-) or nitrous oxide (Schmidt et al, PNAS 1996; 93: 14492). They proposed that NO is derived from NO^- in a reaction catalyzed by superoxide dismutase (SOD). To determine if NOS synthesizes NO, electron paramagnetic resonance (EPR) spectroscopy was applied to directly measure NO formation from purified neuronal NOS. In the presence of the NO trap Fe^{2+}-*N*-methyl-D-glucamine dithiocarbamate, NO gives rise to a characteristic triplet EPR spectrum, with $g = 2.04$ and $a_N = 12.7$ G, whereas NO^- is undetectable. In the presence of L-arginine (L-Arg) and cofactors, NOS generated prominent NO signals. In contrast to previous observations, NO generation did not require SOD. NO formation was blocked by the either the specific NOS inhibitor *N*-nitro-L-arginine methyl ester or by excluding NOS substrate L-Arg. Isotope-labeling experiments with ^{15}N L-Arg demonstrated that NOS-catalyzed NO arose from the guanidino N of L-Arg. Analysis of the time course of NO formation revealed that it paralleled that of the co-product L-citrulline as measured from the conversion of L-[^{14}C]Arg to L-[^{14}C]citrulline. Using the oxygen radical trap DMPO, potent superoxide generation was observed in the reaction system used before. This superoxide was mainly derived from the reaction of NADPH with free FAD and may account for the prior failure to detect NO. Together, these experiments provide the demonstration that purified NOS does directly synthesize NO.

11:00

801-3 Tissue Factor Pathway Inhibitor Release Is Specific for Heparin and Low Molecular Weight Heparins. Implications in the Management of Coronary Syndromes

D. Hoppensteadt, A. Fareed, J. Amiral[1], W. Jeske, J.M. Walenga, B. Lewis, D. Welzel[2]. *Loyola University Medical Center, Maywood, IL, USA; [1]Serbio, Gennevilliers, France; [2]Fredrick Loeffler Strasse, Nurnberg, Germany*

Plaque rupture resulting in the release of on site tissue factor (TF) represents a pivotal event in coronary syndromes. Basal levels of total and free tissue factor pathway inhibitor (TFPI) are strongly influenced by heparinization and a dose dependent increase is observed after both the IV and SC administration of heparins. Plasma samples collected from patients treated with heparin/low molecular weight heparins (LMWHs) and antithrombin drugs (hirudin and argatroban) from various clinical trials (PTCA, thrombolysis, unstable angina) were compared for the relative release of TFPI. Unfractionated heparin and LMWHs produced a dose and route dependent (3–10 fold) increase in the total and free TFPI (up to 30 fold) (Basal levels: free TFPI 9.2 ± 2.4; total TFPI 74.9 ± 9.6, n = 250) in various studies. Recombinant hirudin, hirulog and argatroban at comparable anticoagulant levels in both therapeutic (APTT 70–100 secs.) and surgical/interventional (ACT 400–600 secs.) protocols failed to produce any significant elevation of both forms of TFPI. Additional analysis of these samples for markers of thrombin generation such as prothrombin fragment F 1.2 and thrombin antithrombin complex also revealed that heparin and LMWH patients have significantly lower levels of these markers in contrast to patients treated with antithrombin agents ($p < 0.01$). Since TFPI represents a specific and potent modulator of TF the observed superior efficacy of heparin in coronary syndromes may be attributable to their actions on the release of this inhibitor.

11:15

801-4 Hirudin (Desulfated, 54-65) Causes Contraction of Canine Coronary Arteries Which Is Attenuated by Calcium-Channel Blockers

Paul Sorajja, David G. Cable, Hartzell V. Schaff. *Mayo Clinic, Rochester, Minnesota, USA*

Background: Hirudin is a novel antithrombin agent with potent anticoagulatory properties. Previous studies have demonstrated that hirudin (desulfated, 54-65) stimulates the release of nitric oxide in porcine pulmonary arteries. We investigated hirudin for its direct effects on coronary arteries.

Methods and Results. Canine coronary arteries were mounted in organ chambers and precontracted with prostaglandin $F2_\alpha$ (2×10^{-6} M). In arteries with intact endothelium, hirudin (10^{-10} to 10^{-6} M) increased vascular tension 33.6 ± 9.0% above baseline tension ($n = 10$, $p \leq 0.05$, ANOVA). Similarly, in arteries denuded of endothelium, hirudin increased vascular tension 31.8 ± 11.2% above baseline tension ($n = 8$, $p \leq 0.05$), demonstrating that the hirudin-mediated contractions were endothelium-independent. Pretreatment of the coronary arteries with either verapamil (10^{-4} M) or nifedipine (10^{-4} M) for one hour attenuated hirudin-mediated vascular contractions. Whereas control arteries contracted 26.4 ± 10.3% above baseline tension, hirudin administration increased vascular tension 3.8 ± 7.0% above baseline tension in arteries pretreated with verapamil ($n = 6$, $p < 0.05$ vs. control) and 6.2 ± 12.4% above baseline tension in arteries pretreated with nifedipine ($n = 6$, $p < 0.05$). Contractions to hirudin were also observed in arteries not precontacted with prostaglandin $F2_\alpha$ ($n = 3$), suggesting that the resting tension of the coronary artery was not important in hirudin-mediated vascular responses.

Conclusions. The present study demonstrates that hirudin induces endothelium-independent contractions of canine coronary arteries. Pretreatment with calcium-channel blockers attenuated these contractions, suggesting a possible mechanism (extracellular influx of calcium) by which hirudin mediates coronary artery contsriction.